Pedobiologia **27**, 261—267 (1984)

Section of Forest Ecology, Department of Ecology and Environmental Research & Department
of Chemistry and Molecular Biology,
Swedish University of Agricultural Sciences, Uppsala

Dynamics of some nitrogen fractions in decomposing Scots pine needle litter

BJÖRN BERG and OLOF THEANDER

With 2 figures

(Accepted: 84-03-21)

1. Introduction

The release of nutrients from litter and humus is a fundamental process in the biogeochemical cycle and its rate is an important factor for plant growth. Nitrogen often appears to be limiting both plant growth (TAMM 1964) and microbial processes in northern forest soils (BERG & STAAF 1980); but mobilization mechanisms from dead organic matter are in no way fully understood at present. Among suggested regulating factors is the ratio between the total carbon and nitrogen supplies (C/N-ratio) for litter. With ratios above a certain value no mobilization would take place. These critical values vary considerably (BERG & EKBOHM 1983); however, and the litter's mass loss rate itself is probably also involved as a factor (BERG & STAAF 1981).

The organic-chemical composition of litter will influence both its rate of decomposition and release of nutrients. The level of lignin, for example, could have this effect (BERG & STAAF 1980). Lignin itself, or rather lignin and other components analyzed as Klason (sulfuric acid) lignin, is the last of the litter's organic chemical components to be degraded (BERG et al. 1982) and when its degradation starts it appears to be rate-determining for litter decomposition (BERG & STAAF 1980). Earlier lignin has been considered to be a slowly degraded complex but apparently it can be degraded at different rates (BERG, WESSÉN & EKBOHM 1982) which possibly are inversely related to the litter's level of total nitrogen.

It is known that the concentration of nitrogen increases in litter under decomposition (e.g., HAYES 1965, ABER & MELILLO 1979, BERG & STAAF 1981) at least in the decomposition stages so far observed. Also the absolute amount of nitrogen compared to the initial amount bound to, e.g., needle litter, may increase during decomposition — see a review by BERG & STAAF (1981). This net immobilized nitrogen may reach amounts up to three times the original amount (HAYES 1965). Also the amount of nitrogen associated with the analytical Klason (sulfuric acid) lignin fraction has been found to increase and FLAIG *et al.* (1959) noticed an increase in the concentration of nitrogen bound to both milled wood lignin and sulfuric-acid lignin.

The aim of the present work was to describe the changes in total nitrogen with special reference to the changes of nitrogen associated with the sulfuric-acid lignin fraction in the needle litter in a long-term study of decomposition, and relate it to the system studied. Part of this work has been presented earlier (STAAF & BERG 1982).

2. Site description

The 120- to 130-year-old Scots pine stand studied is located at the SWECON research site, Ivantjärnsheden, Central Sweden (60°,49′ N, 16° 30′ E) at an altitude of 185 m above m.s.l. on a flat area of deep glacifluvial sand sediments. The mean annual precipitation at a nearby village is 609 mm (1931-60), and the mean annual temperature +3.8 °C. The length of the growing season is about 160 days (i.e. number of days with a daily temp. higher than 6 °C; Axelsson & Bråkenhielm 1980).

The tree layer is composed exclusively of *Pinus silvestris* L. and has a density of 393 trees per ha and a height of 17—19 m. *Calluna vulgaris* Hull, and *Vaccinium vitis-idaea* L. form a well-developed field layer, and the bottom layer, completely covering the ground, is mainly composed of the mosses *Pleurozium schreberi* (Brid.) Mitt. and *Dicranum polysetum* Sv. together with *Cladonia lichens.* The most recent direct effect of forestry practices was a slight thinning in 1960. A more complete description is given by Axelsson & Bråkenhielm (1980).

The soil profile is an iron podsol with a weakly developed bleached horizon (2—7 cm) and the humus type is a typical mor. A very loose litter layer (A_{00}), interwoven with living mosses and lichens, covers a humus layer (A_{01}—$_{02}$) of 5—10 cm. The A_{01} (F) and A_{02} (H) horizons are almost indistinguishable from each other. The pH range is 3.9—4.2 in the humus layer and 4.6—4.8 in the upper mineral soil. The parent mineral material as well as the whole soil is considered to be very poor in essential nutrients. The C/N ratio of the A_{01}-A_{02} layer is 42.3.

3. Materials and methods

3.1. Needle collection, storage and sample preparation

Brown needles were sampled at Ivantjärnsheden in late October 1973, and taken from the branches of about 15-year-old Scots pines at abscission, air dried and were stored at —20 °C until the sample preparation took place. The trees were all located in an area less than 20 × 50 m². Before weighing, the needles were dried at room temperature to reach an even moisture level (6.94 %). The biggest difference in moisture was less than ± 0.5 % from the average as determined on 20 samples.

The litter-bags measuring 8 × 8 cm, were made of terylene net with a mesh size of 1 mm. Each litter-bag contained about 2 g of needle litter and a piece of plastic tape giving the mass of the needles. The litter-bags were placed on the A_{00} layer at 20 test plots (1 × 1 m²) randomly selected in the 120-year-old stand (3 ha) in spring as soon as the snow had melted (May 12, 1974). Five hundred litter-bags were incubated. The bags were fastened to the ground by 10—15 cm long metal pins and were incubated for 123, 185, 344, 376, 490, 545, 726, 733, 845, 922, 1125, 1228, 1273, 1480, 1581, 1627, 1825 and 1966 days.

3.2. Sampling and chemical analysis

For mass loss determinations one sample from each of the 20 test spots was collected, transported directly to the laboratory and cleaned of mosses, lichens and cowberry remnants. At some of the samplings half a needle was taken out per sample for determination of invading mycelium (Berg & Söderström 1979). After the removal of plant remains, the loss of dry mass of the needles was determined by drying the aliquots to constant mass at 85 °C. At the low pH (below 4.0) no measurable volatilization of NH_3 should be expected at this temperature (H. Nömmik, pers. comm.). The nitrogen determinations were done by Kjeldahl analysis and the ash contents by heating at 600 °C for 3 h.

After extraction (Berg *et al.* 1982) the dried residues were analysed for sulphuric acid (Klason) lignin by hydrolysis [12.0 *M* H_2SO_4 at room temperature for 2 h and reflux for 6 h after dilution to 0.358 *M* (Bethge *et al.* 1971)].

4. Results and discussion

4.1. Comments on the fractions studied

We have used two ways of describing the change of nitrogen, either as a concentration (%) of the sampled litter (relative amount) or as a change in net amount calculated from a certain initial quantity (absolute amount). The latter is given as mg per initial g of litter. Both these values are given in Figure 1. The difference between total nitrogen and that analyzed as associated with the sulfuric-acid lignin is given as "non-lignin nitrogen" (Fig. 2).

The term "lignin" is not fully correct in connection with decomposing plant litter. We have preferred to use it though, for the sake of simplicity.

One would expect that the residue (fraction) analyzed as sulfuric-acid lignin not only represents native or modified lignin but also originates from other phenolic compounds (such as tannins), cutins, carbohydrates (furan and phenolic degradation products) and various nitrogenous compounds. In one of the authors' laboratory the formation of phenolic and other aromatic compounds was demonstrated to derive from degradation of carbohydrates alone or in the presence of amino compounds by heat treatment under mild pH-

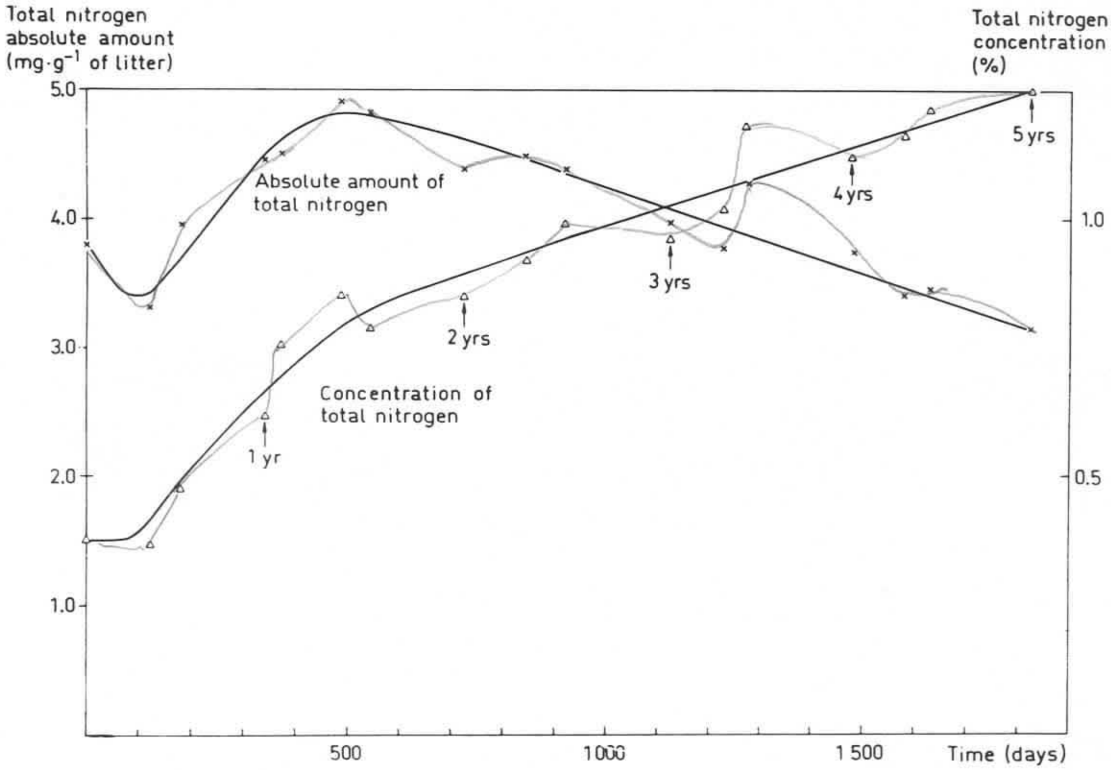

Fig. 1. Changes in relative amount (concentration) and absolute amount of nitrogen in decomposing Scots pine needle litter during decomposition over a five-year period.

conditions (the Maillard reaction-for a recent summary, see Theander 1980). One would expect similar reaction products and their condensation products to be slowly formed also with the litter conditions prevailing in the present study. A comparison could be made with a study on *Sphagnum* moss and *Sphagnum* peat of different ages (Theander 1954). In this it was found that the increase of non-carbohydrate polymers originated mainly from carbohydrate degradation and lipids (Ekman & Ketola 1981) as the original amount of lignins or lignin-like compounds in the original mosses was very low (Lindberg & Theander 1952). In a more recent investigation on decomposition of *Sphagnum* moss there was an increase of the absolute amount of sulfuric-acid lignin from initially 40 to about 100 mg g^{-1} of litter after two years of incubation (Berg 1984).

The nitrogen in the sulfuric-acid lignin fraction may originate from pine needle cell-wall protein, protein-tannin (or other polyphenols) complexes, slowly formed Maillard reaction products (Theander 1980) but also from microbial chitin, and nucleic acids. In this connection it is of interest to note that a brown humic product, insoluble in water, which was formed by treating an aqueous solution of glucose and glycine at 96 °C (Olsson, Pernemalm & Theander 1978) analyzed for 90 % Klason lignin by the standard procedure used in the present investigation.

The nitrogen bound to fungal biomass was not measured separately in the present study but measurements from the first three years are given here from a previous presentation (Berg & Söderström 1979).

4.2. Total nitrogen fraction

It appears possible to distinguish three separate phases for the changes in absolute amount of total nitrogen taking place during decomposition, namely leaching, accumulation (net immobilization) and release (net mineralization) phases (Fig. 2). These are commonly seen, even if leaching or accumulation may sometimes be difficult to distinguish (Berg & Staaf 1981).

The absolute amount of nitrogen dropped during the first months of decomposition and about 10 % of the initial amount of nitrogen left the nedeles, probably mainly by leaching

(Fig. 1). This process often releases rather large amounts of nutrients (Bogatyrev *et al.* 1983), including those present in carbon compounds from leaf litter (Nykvist 1963).

The absolute amount of total nitrogen (Fig. 2) — after the initial loss due to leaching — increased considerably during the first 500 days up to about 130% (C/N $\sim$70), after which it slowly decreased. This accumulation followed the type function $1\text{-}e^{-x}$ *versus* mass loss well with $p < 0.001$ ($n = 5$, $r = 0.966$) but could be approximated to a straight line ($p < 0.001$). Accumulation of this kind has been found to take place in very different ecosystems and the magnitude appears to depend on both system and litter type (Berg & Staaf 1981). In their work (Berg & Staaf 1981) they found empirically that the critical N-concentration fo release to take place partly depends on the decomposition rate — higher N-levels found at higher rates.

When the release had started the absolute amount of nitrogen did not go below the initial amount until after four years. The decrease in amount from 500 days onwards was linear ($p < 0.001$) for nitrogen release *versus* mass loss ($n = 11$, $r = 0.927$, $p < 0.001$). We may note that the net release of nitrogen started at about the same time as the decomposition of lignin.

The concentration of total nitrogen increased with time (0.38 to 1.20% during the five years) (Fig. 1). With the exception of the first year, the samples collected in spring had a comparatively low concentration whereas it was considerably higher in October/November. The highest increases in concentration thus took place in the same period as the high litter decomposition. This relative increase plotted *versus* mass loss also gave a highly significant straight line relation ($p < 0.001$; *cf.* Staaf & Berg 1982). The increase in concentration continued in spite of a net release from the litter (Fig. 1).

The absence of soil animals participating in the decomposition is essential for distinction of the phases discussed. Whereas the microbial breakdown only removes CO_2, soil animals will remove whole pieces of litter including nutrients and thus disturb the quantification of nitrogen. In the stand studied, the soil fauna appears to be of little significance for litter decomposition (Berg *et al.* 1980).

4.3. Nitrogen bound to the "lignin" fraction

There was a notable relative increase of nitrogen in the "lignin" fraction and the concentration increased during the whole five-year period and became about doubled (0.44 to 1.0%) (Table 1). This increase in concentration with time followed the type function $1\text{-}e^{-x}$ rather closely ($p < 0.001$) and a linear relation was thus obtained between the concentration of "lignin nitrogen" and accumulated litter mass loss ($p < 0.001$).

The absolute amount of "lignin-nitrogen" increased as decomposition proceeded until a net mass loss of the "lignin" fraction started at 700 to 800 days (Fig. 2; Table 1). By then the amount of nitrogen bound to this fraction was almost doubled. In litter the decomposition of "lignin" is slower than for the other organic chemical components and starts late (Berg *et al.* 1982), and the release of "lignin-nitrogen" thus started later (after about 900 days) than the release from the total-nitrogen fraction (500 days, Fig. 2) and a clear release was not seen until after 4 years (Fig. 2). In a recent review Nömmik & Vaathras (1982) discussed mechanisms for the uptake of NH_3 to, among others, polyphenolic substances. It was suggested by Mattson & Andersson (1942) that phenolic hydroxyl groups are the sites where the NH_3 is bound.

The accumulation in lignin may be described as a temporary sink for nitrogen at least in the early stages if decomposition. This fixed nitrogen appears to be, at least partly, available to mineralization and uptake by plants as found by Burge & Broadbent (1961).

4.4. Nitrogen not bound to the "lignin" fraction

The nitrogen not bound to the "lignin" fraction was not determined by direct analysis but was calculated as a difference between the total nitrogen and the "lignin-nitrogen" fractions. The chemical compounds making up this nitrogen fraction probably are nucleic

Table 1. Litter mass loss, concentration of lignin in litter and changes in concentration and absolute amount of nitrogen as well as ash in the sulfuric-acid fraction of decomposing Scots pine *(Pinus silvestris)* needle litter

Incubation time (days)	Litter mass loss[1]) (%)	S.D.	Lignin concentration in litter (%)	"Lignin nitrogen" concentration (% in lignin)—	"Lignin nitrogen" Absolute amount (mg g^{-1} of litter	Ash concentration in lignin (%)
0	*0*	—	*25.0*	*0.44*	1.10	*0.84*
123	*10.4*	1.02	*27.8*	*0.50*	1.24	
185	*17.8*	3.98	*28.3*	*0.61*	1.42	
344	*24.4*	4.47	*33.2*	*0.74*	1.86	*0.98*
376 (1 yr)	*27.3*	5.19	*36.1*	*0.74*	1.94	
490	*35.7*	4.07	*38.1*	*0.74*	1.81	*1.19*
545	*43.2*	4.70	*41.6*	*0.72*	1.70	
726	*44.4*	3.71	*42.6*	*0.75*	1.78	
733 (2 yr)	*45.8*	5.55	*42.4*	*0.78*	1.82	
845	*51.2*	5.55	*42.7*	*0.79*	1.65	*1.47*
922	*55.8*	5.32	*46.3*	*1.02*	2.09	
1125 (3 yrs)	*58.8*	7.38	n.d.	n.d.	n.d.	
1228	*63.0*	5.18	*45.4*	*1.05*	1.76	
1273	*63.8*	3.94	*44.3*	*0.94*	1.51	
1480 (4 yrs)	*66.5*	5.68	*46.0*	*0.98*	1.51	
1581	*70.8*	7.20	*48.1*	*1.09*	1.53	*2.71*
1627	*71.4*	6.93	*47.1*	*1.01*	1.36	
1825 (r yrs)	*75.0*	4.16	*47.0*	*0.97*	1.14	
1966	*77.5*	8.14	*47.2*	*1.04*	1.12	

[1]) Given earlier by Berg *et al.* (1982)
Note: n.d. stands for "not determined"

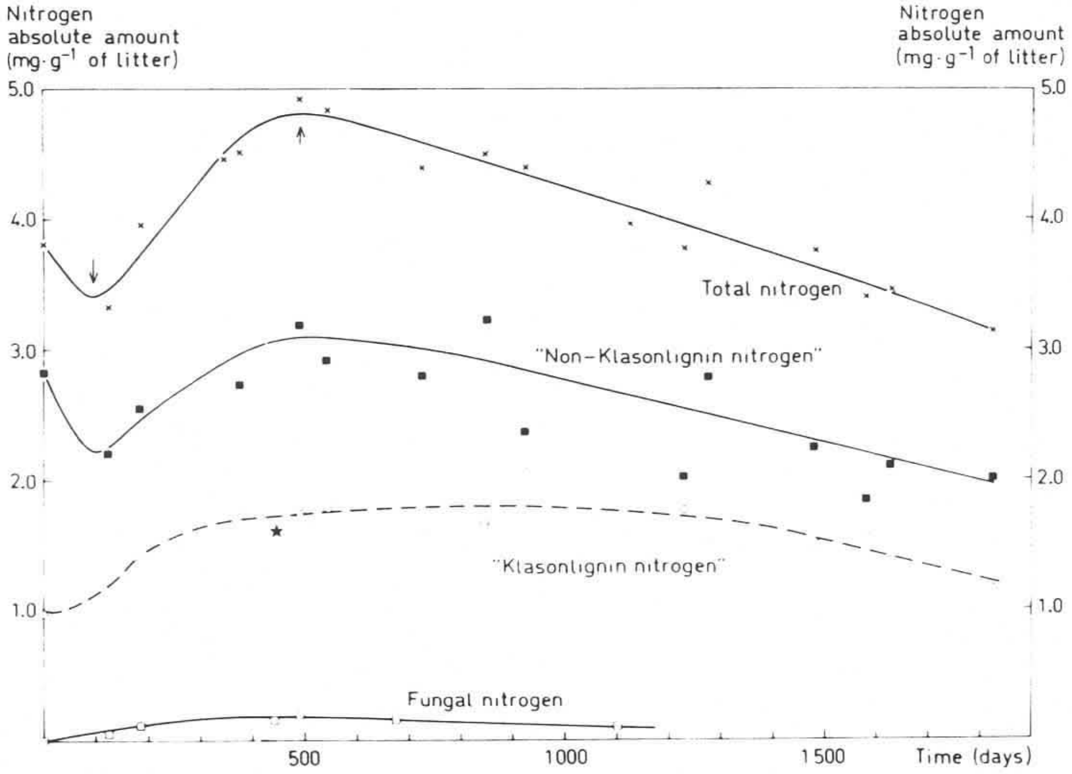

Fig. 2. Changes in absolute amounts of some nitrogen fractions in decomposing Scots pine needle litter over a five-year period. The star marks the time when a net decomposition of the lignin fraction started. The arrows in the graph for total nitrogen indicate the suggested start of accumulation and release phases.

acids and proteins originating from both the plant and invading microorganisms (Fig. 2) This nitrogen pool probably should be more easily available to microorganisms than that bound to "lignin". After the initial decrease caused by leaching, this pool increased somewhat and its behaviour was similar to that of total nitrogen because after the first year "lignin-nitrogen" appeared to be relatively stable.

5. Acknowledgements

The skilful technical assistance of Ulla Larsson and Seved Helmersson is gratefully acknowledged. Dr. Håkan Staaf is gratefully acknowledged for many valuable discussions.

The investigation was performed within the Swedish Coniferous Forest Project supported by the Swedish Natural Science Research Council and Swedish National Environmental Protection Board.

7. References

Aber, J., & J. M. Melillo, 1979. Litter decomposition: measuring relative contributions of organic matter and nitrogen to forest soils. Can. J. Bot. **58**, 416—421.

Axelsson, B., & S. Bråkenhielm, 1980. Investigation sites of the Swedish Coniferous Forest Project — Biological and Physiological Features. In: Persson, T., (ed.), Structure and Function of Northern Coniferous Forests — an Ecosystem Study. Ecol. Bull. (Stockholm) **32**, 25—64.

Berg, B., 1984. Decomposition of moss litter in a 120-year-old Scots pine forest. Pedobiologia **26**, 301—308 (in press).

— & G. Ekbohm, 1983. Nitrogen immobilization to decomposing needle litter at variable carbon: nitrogen ratios. Ecology **64**, 63—67.

— & B. Söderström, 1979. Fungal biomass and nitrogen in decomposing Scots pine needle litter. Soil. Biol. Biochem. **11**, 339—341.

— B. Wessén & G. Ekbohm, 1982. Nitrogen level and decomposition in Scots pine needle litter. Oikos **38**, 291—296.

— K. Hannus, T. Popoff & O. Theander, 1982. Changes in organic-chemical components of needle litter during decomposition. Long-term decomposition in a Scots pine forest I. Can. J. Bot. **60**, 1310—1319.

— U. Lohm, H. Lundkvist & A. Wirén, 1980. Influence of soil animals on decomposition of Scots pine needle litter. In: Persson, T. (ed.) Structure and Function of Northern Coniferous Forests — an Ecosystem Study. Ecol. Bull. (Stockholm) **32**, 401—409.

— & H. Staaf, 1980. Decomposition rate and chemical changes of Scots pine needle litter II. The influence of chemical composition. In: Persson, T. (ed) Ecol. Bull. (Stockholm) **32**, 373—390.

— & H. Staaf, 1981. Leaching, accumulation and release of nitrogen in decomposing forest litter. In: Clark, F. E. & Rosswall, T. (eds) Terrestrial Nitrogen Cycles — Processes, Ecosystem Strategies and Management Impacts. Ecol. Bull (Stockholm) **33**, 163—178.

Bethge, P. O., R. Rådeström & O. Theander, 1971. Kvantitativ kolhydratbestämning — en detaljstudie. Comm. from Swedish Coniferous Project Research Lab. Stockholm 63B. (In Swedish).

Bogatyrev, L., B. Berg & H. Staaf, 1983. Leaching of plant nutrients and total phenolic substances from some foliage litters — a laboratory study. Swedish Conif. For. Proj. Technical Report **33**, 25 pp.

Burge, W. D., & F. E. Broadbent, 1961. Fixation of ammonia by organic soils. Soil Science Soc. of Amer. Proc. **25**, 199—204.

Ekman, R., & M. Ketola, 1981. Analysis of lipid components in peat from a Finnish *Sphagnum* bog. Kemia-Kemi **8**: 488—493.

Flaig, W., U. Schobinger & H. Deuel, 1959. Umwandlung von Lignin in Huminsäuren bei der Verrottung von Weizenstroh. Chem. Ber. **92** (9), 1973—1982.

Hayes, A. J., 1965. Studies on the decomposition of coniferous leaf litter. 1. Physical and chemical changes. J. Soil Sci. **16** (1), 121—140.

Howard, P. J. A., & D. M. Howard, 1974. Microbial decomposition of tree and shrub leaf litter. 1. Weight loss and chemical composition of decomposing litter, Oikos **25**, 341—352.

Lindberg, B., & O. Theander, 1952. Studies on *Sphagnum* peat II. Lignin in *Sphagnum*. Acta Chemica Scandinavica **6**, 311.

Mattson, S., & E. Andersson, 1942. The acid-base condition in vegetation, litter and humus: V. Products of partial oxidation and ammonia fixation. Ann. Agr. Coll. Sweden **10**, 284—332.

Nykvist, N., 1963. Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Studia Forestalia Suecica **3**, 1—31.

Olsson, K., P.-E. Pernemalm & O. Theander, 1978. Formation of Aromatic Compounds from Carbohydrates. VII Reaction of D-Glucose and Glycine in slightly acidic aqueous solution. Acta Chemica Scandinavica. **32**, 249—256.

Staaf, H., & B. Berg, 1982. Release and accumulation of plant nutrients in needle litter during decomposition. Long-term decomposition of Scots pine needle litter II. Can. J. Bot. **60**, 1561 to 1568.

Tamm, C. O., 1964. Determination of nutrient requirements of forest stands. In: Romberger, J. A., & P. Mikola, (eds) International Review of Forestry Research, vol. 1, pp. 115—170. New York-London: Academic Press.

Theander, O., 1954. Studies on *Sphagnum* peat. III. A Quantitative study on the carbohydrate constituents of *Sphagnum* mosses and *Sphagnum* peat. Acta Chemica Scandinavica 8, 989 to 1000.

— 1980. Sugars in thermal processes. In: P. Koivistonen & L. Hyvönen (eds), Carbohydrate sweeteners in foods and nutrition. pp. 185—199. Academic press, London.

Addresses of the authors: Björn Berg (corresponding author), Avdelningen för Skogsekologi, Institutionen för Ekologi och Miljövård, Sveriges Lantbruksuniversitet [Section of Forest Ecology, Department of Ecology and Environmental Research, Swedish University of Agricultural Sciences], S - 750 07 Uppsala, Sweden. Olof Theander, Institutionen för kemi och molekylärbiologi. Sveriges Lantbrukuniversitet [Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences], S - 75007 Uppsala, Sweden.

6. Synopsis *Original scientific paper*

Berg, B., & O. Theander, 1984. Dynamics of some nitrogen fractions in decomposing Scots pine needle litter. Pedobiologia **27**, 261—267.

Changes in absolute amounts of two nitrogen fractions namely total nitrogen and nitrogen bound to sulfuric-acid lignin respectively, were followed in decomposing Scots pine *(Pinus silvestris)* needle litter for five years down to a litter mass loss of 75%. A net immobilization of nitrogen took place during the first $1^1/_2$ years and up to 130% of the initial amount. This was then followed by a release. In the net immobilization and release phases the uptake and release respectively were in proportion to litter mass loss. The concentration of nitrogen increased in proportion to litter mass loss ($p < 0.001$) There was also a net increase of nitrogen bound to the sulfuric acid lignin fraction and the absolute amount of nitrogen was almost doubled. A release of this nitrogen did not start until after 4 years.

Key words: Litter, pine needles, decomposition, lignin, nitrogen, mineralization, immobilization.